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COMPARISON OF BIOCHEMICAL FINDINGS AND CLINICAL PICTURES ON SUBACUTE OR CHRONIC ARSENIC POISONING OF INFANTS DUE TO ARSENIC-CONTAINING POWDERED MILK

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HISO KONNYU FUNNYU NI YORU NYUJI AKYUSEI NAISHI MANSEI HISO CHUDOKU NO SEIKAGAKUTEKI SHOKEN TO RINSHOZO TONO TAIHI [COMPARISON OF BIOCHEMICAL FINDINGS AND CLINICAL PICTURES ON SUBACUTE OR CHRONIC ARSENIC POISONING OF INFANTS DUE TO ARSENIC-CONTAINING POWDERED MILK]

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COMPARISON OF BIOCHEMICAL FINDINGS AND CLINICAL PICTURES ON SUBACUTE OR CHRONIC ARSENIC POISONING OF INFANTS DUE TO ARSENIC-CONTAINING POWDERED MILK

Hideo Nagai, Tomofusa Usui, Akira Asahi, Minoru Takata,
Zenzaburō Takenaka, Hideo Tōmura, Shigeru Miki, Kazuo Koike,
Shoji Hayakawa and Hisakazu Wada

Department of Pediatrics, Kyoto University Medical School
(Chief Professor: H. Nagai)

The report [1] announced by our department concerning the arsenic poisoning of infants which occurred last summer dealt mainly with the clinical picture. When this detailed report is compared to the scattered reports of cases of arsenic poisoning since the 1800's, we find that this arsenic poisoning possesses extremely peculiar characteristics. That is:

1) Arsenic was contained in the powdered milk which is the major nutritional source for infants. Hence, the patients were infants.
2) For the cases concerned, the quantity of arsenic was not large enough to cause acute arsenic poisoning.
3) A relatively small quantity of arsenic was taken over a long period of time.
4) The powdered milk containing arsenic was consumed during the summer.

The reports of arsenic poisoning appearing in the bibliography are primarily as follows. Most cases concerned adults; only Woody [2] had a case of a 6-months old infant. The largest number of cases occurred in Liverpool [3] in 1901 when 6000 people were affected by beer poisoning; 71 of them died. In Weingenuss poisoning in Toulon in 1888, 500 were affected, and 15 of them died. However, the reports with a large number of cases — i.e., poisoning symptoms were definite — were almost all acute poisoning. For chronic

poisoning, there is an interesting report by Hughes [4] in which a 59-year old woman had taken 1.7 mg of As_2O_3 everyday for 17 years.

Thus, despite the fact that there are many reports on arsenic poisoning, reports dealing with arsenic poisoning which occurred here are extremely rare. Although the various biochemical studies were conducted without having sufficient preparatory knowledge because of a sudden occurrence of this incident, these are gradually being substantiated by zoological experiments which have been performed since then. Therefore, we shall report on some of the findings here.

Arsenic poisoning, as seen from a biochemical examination, is treated in this paper with particular emphasis on the mutual relation between the degree of each clinical symptom and the quantity of arsenic intake in order to establish its relation to clinical observations.

I. TOXICITY OF ARSENIC DERIVATIVES

A) Kinds of Arsenic Derivatives

The structure of arsenic derivative in powdered milk is quite complex ($2\ Na_3(PAsO_4)Na(FOH).22H_2O$). An accurate toxitity test of this complex salt, which is difficult to synthesize, is impossible today. In any event, arsenic compounds seen in arsenic poisoning have the following order of decreasing toxicity: ordinary arsenious acid, arsenic acid, arsenite, and arsenate.

According to A. Heffter [5], arsenic derivative can be classified as follows:

I. Inorganic arsenides
   1) Arsenic hydrogen (AsH_3)
   2) Solid arsenic compounds arsenious acid (As_2O_3) and arsenic acid $\{H_3AsO_4\ (2H_3AsO_4+H_2O)\}$

II. Organic arsenides
   1) Arsines of the aliphatic chain

2) Arsines of the aromatic chain
3) Arsine acid of the aliphatic chain
4) Arsine acid of the aromatic chain

B) Toxicity of Arsenic Derivatives

Such arsenic compounds have at least some toxicity against animals. We shall touch a little on the toxic action next. The following conclusions may be stated based on what is known today:

"The toxic action of arsenic results from the combination of cells with certain vital components. Further, this combination is reversible in a living body."

Among the vital components in these cells, the SH enzyme is obvious, and arsenic and SH which exists within an active group of these enzymes form a thiol combination and cause degradation in the function of these enzymes and a metabolic impediment. The kind of enzymes which are affected is simply stated as follows:

1) Experiment in a test tube:
   i) Enzymes which are impeded
      Protease (tyrpsin, erepsin, kathepsin)
      Impedes ($^{\text{in vitro}}_{\text{in vivo}}$) the reduction of oxayl acetic acid, dehydrogenase, fumaric acid
      Esterase, dehydrogenase, xanthinoxydase, etc.
      Generally impedes oxydation and reduction processes
   II) Enzymes which are promoted

2) Experiment in a living body
   Reduction in synthesizing ability of muscles and reduction in concentration of hexosemono phosphorous acid (rabbit)
   Glykolyse: promotion or impediment depending on concentration; also depends on the kind of animal species and the kind of arsenic derivative

Impedes amylase

Reduction in basal metabolism and Rest-N (human beings, administered once)
In the chronic administration, a rise in Rest-N and an increase in protein disintegration

However, the results may be opposite depending on the individual. It also depends on the species of animal, quantity of arsenic, kind of arsenic derivative, and the method of administration. Thus, its toxic action can not be simply explained. Ultimately, arsenic affects the cellular respiration and causes changes in protein, fat, and sugar metabolism; consequently, it becomes an attacking substance in many places in the metabolic process. Heffter concluded that the toxic action results from an invasion of the enzyme process, which is necessary for life. The effects of arsenic against enzyme action can be seen in a reduction of thyroid gland activity, and a reduction in the thyrotrophic hormone secretion of the hypophysis prothallium, or against bone tissue, in an increase in the density of long bones, a multiplication of cartilage cells, and a change of spongiosis (rabbit). Also, an increase in the cell division of the lymph glands (guinea pig, dog), and teratoma in bones and connective tissues (chicken) sometimes occur. Acceleration and multiplication (a minute quantity of arsenic) (chicken) of the tissue culture of spleen and iris can be recognized. In liver, cirrhotic changes (around the portal vein), biliary dust impediment, and paralysis or a contractile and atonic action of blood vessels may be found. In a digestive tract, intestinal vermiculation increases (rabbit) and the gastric juice secretion is also affected. The lymphatic tissues are also strongly affected and the impediment of reticular tissues of the lymphatic gland, the spleen, and the medulla is seen. Impediments in blood and skin and agranulocytose, acute yellow liver atrophy are also seen. The above toxic action is mainly based on zoological experiments, and thus can not be immediately applied to human beings, but it is useful in making some estimates. We have not yet encountered any report which goes into this level of detail in the examination of poisoning cases on human beings.

C) Amount of Toxicity of Arsenic Derivative

The normal quantity of arsenic in a living body as well as the quantity of toxicity have been studied in the literature, and the following is obtained.

TABLE 1. AMOUNTS OF As IN ORGANS OF NORMAL ADULT

Organ	Amounts of As γ%
Liver	11.1
Cerebrum	10.6
Spleen	8.8
Brain	11.1
Heart	9.9
Lung	9.5
Thyroid Gland	13.1
Bone	8.8
Nail	17.2
Hair	9.7
Blood	83.0
Urine	8.0

i) Normal quantity: According to Biol. Daten. f. Kinderarzt [6], the representative data are as shown in Table 1. The fairly substantial quantity of arsenic is contained in a living body and is fulfilling some important physiological roles.

ii) Toxic quantity: In the case of a misuse of the arsenic derivative or use with the purpose of committing suicide, the quantity of arsenic in various internal organs is quite high Z.B. Heffter 1927. However, to determine the toxic quantity for human beings is difficult, because cases in which a quantity of arsenic intake is clearly known corresponding to the given symptoms are very rare. Thus, the mutual relationship between the quantity of arsenic and the poisoning symptoms is difficult to judge, even though the lethal dose can be estimated to some degree.

Table 2 was compiled by Heffter [7] who collected data from the zoological experiments. The lethal dose may be considered to be about 10 mg As_2O_3/kg per intake. The difference between lethal dose and non-lethal dose is extremely small. Hence, it is a dangerous toxic material when an individual difference is considered. Heffter [5] observed that even a small quantity becomes lethal, if an animal is fed insufficient vitamins. According to the report by Woody, N.C. [2], even 1.0 mg/kg of arsenic can become lethal in the case of an infant.

If a minute quantity is taken over a long period of time, as in the case of arsenic poisoning due to MF dried milk, the lethal dose is not known today. We obtained 18 cases in which the quantity of arsenic intake is clearly determined, but fortunately no death occurred; hence, no knowledge on its lethal dose was obtained. Thus, the report of Hughes, R.R., mentioned previously becomes the only reference concerning this.

II. ON THE DETERMINATION OF ARSENIC

There are many methods of determination, but based on Jacobs, M.B. [8], and Sandell, E.B. [9], the major ones are as follows:

1) Reinsch Test
2) Gutzeit Method (also, a modified method by Goldstone)
3) Molybdenum Blue Method
4) Bromate Method
5) Other Methods (Iodometric Method, Chlorometric Method, Polarography)

Among the above, the one with the best sensitivity is the Gutzeit method, and the one with a relatively good sensitivity and accuracy is the Molybdenum Blue method. We conbined these two, using the Goldstone method, which is an improvement over the Gutzeit method, and changed the Molybdenum Blue method somewhat to triple its sensitivity. These methods are briefly described below.

1) Goldstone Method (Its principle is identical to the Gutzeit method)

TABLE 2. TOXITY OF As_2O_3 TO VARIOUS KIND OF ANIMALS

Kind of Animals	Kind of Administrations	Exact Lethal Dose As_2O_3 gm/kg	Non Exact Lethal Dose As_2O_3 gm/kg	Author
Rabbit	Oral	0.03 (Solut)	0.02	Brouardel
		0.01 (〃)	0.005 (Solut)	de Bussher
		0.02 (Solid)	0.015~0.012 (Solid)	〃
		0.016~0.02 (Solut)		Willberg
		0.014 (Solut)	0.013 (Solut)	Sieber
	Sub-cutaneous	0.01		Brouardel
		0.01	0.007~0.009	Morishima
		0.007	0.005~0.006	Sieber
	Intra-venous	0.007		Brouardel
		0.0065	0.006	Morishima
		0.007	0.006	Kochmann
		0.007	0.006	Sieber
Dog	Oral	0.0075 (K. arsenic)	0.005 (K. arsenic, Chronic poisoning)	de Bussher
		0.03< (Solid)	0.02~0.03	〃
		0.07	0.06	Rouyer
	Sub-cutaneous	0.006		Willberg
	Intra-venous	0.005		〃
		0.003		Rouyer
GuineaPig	Oral	0.02~0.03		Brouardel
		0.013		〃
		0.0068		Willberg
	Intra-Peritoneal	0.016		Brouardel
Cat	Sub-cutaneous	0.0047		Willberg
Mouse	〃	0.011~0.013		Willberg
Hedgehog	〃	0.016	0.008	〃
Cock	Oral	0.06~0.15 (Solid)	0.1~0.15 (Solid)	Hausmann
				Willberg
	Sub-cutaneous	0.015		Reich
Horse	Oral	3.75 (Solut)		Hering

This method is simple and yet extremely sensitive (0.1 γ). However, even if the shape and the capacity of the device are completely identical, it is difficult to discharge the gas at a constant velocity always. The stuffing

of glass wool (raw cotton) and sand also can not be strictly constant. Further, when a constant-width filter paper impregnated with mercuric bromide is suspended perpendicularly in a glass tube with a 2.6-2.7 mm inside diameter, a completely identical condition is difficult to establish. Consequently, even if a contrast is always to be taken, the length and the tone of the stain will not be constant. Furthermore, when the standard filter paper is made separately for many steps at a constant amount of As_2O_3 and compared with this*, about 0.2 γ error often exists. Also, if the amount of arsenic becomes more than 10 γ, a judgment is difficult. Therefore, after paying meticulous attention and strictly performing a contrast, the average of the determinations made by three persons was computed and used as the data. The standard filter papers were kept in a darkroon, but they were made anew every 2-3 days.

2) Molybdenum Blue Method

Compared to the former method, this has a lower sensitivity (about 3 γ). However, the data are accurate, because the final color is measured electrophotometrically. We reduced each reagent and raised the sensitivity to 1 γ in order to increase the terminal concentration. Evaporated AsH_3 dissolves in bromine water extremely well, and AsH_3 can be completely absorbed at 3 cc if there are sufficient glass balls.

The most important item to be remembered in both operations is to quickly insert a stopper after inserting active zinc. It is also important not to include arsenic in the reagents at all. For this purpose, only the highest quality reagents are used. Active zinc used is made by Merck. However, the one made by Katayama can also be used.

The above two methods correspond to the final stage in the actual determination (biomaterial) and, before these operations, sample breakdown is performed.

*Translator's note: The original text is unclear on what "this" is referring to.

As this breakdown controls the success of the arsenic determination, we worked hardest on this stage. In this regard, the following precautions should be observed:

i) As an oxidizing agent, among nitric acid, sulfuric acid, perchloric acid, and hydrogen peroxide, it is better to add only nitric acid and sulfuric acid several times.

ii) Before an oxidizing agent is to be added to the sample, it should always be cooled.

iii) There is a loss of arsenic if the color blackens during the oxidation.

iv) The temperature during the oxidation should be low at first, but one can be raised after the white fumes of SO_3 leave. However, if large bubbles or boiling result, then a loss of arsenic occurs.

v) If a great deal of sulfuric acid is used, the concentration takes time after adding water finally. Thus, it is better to make the quantity small (2-5 cc) and add nitric acid several times.

vi) If nitric acid remains even after the solution becomes colorless or light yellow, nitric acid gas is generated in large quantity during the later distillation process and an arsenic determination sometimes becomes impossible. Therefore, we performed (using aniline) a nitric acid ion reaction using the solution and confirmed that there was no influence on the results while colorless, or up to a pink coloration. The solution finally made was filled up to 25 cc, and 5 cc was determined by the Gutzeit method and 20 cc was determined by the Molybdenum Blue method and their average value was taken.

vii) Blanks should be run every time on all reagents used.

1 gm of powdered milk, 5 cc of blood, 50 cc of urine, and 1 gm of feces (dry weight) were used for the test, while the entire samples of hair, nail, blister fluid, and pustule fluid were used.

III. RELATION BETWEEN AMOUNT OF ARSENIC AND CLINICAL SYMPTOMS

1) Relation Between Amount of Arsenic Intake and Clinical Symptoms

Table 3 shows a comparison between the clinical symptoms and the total amount of arsenic intake, relatively accurately computed from an arsenic determination on powdered milk in 14 cases. The total arsenic intake is expressed per kilogram of patient body weight, resulting in one group of 7 cases in which the total arsenic intake was more than 10 mg/kg and the other group in which the total arsenic intake was less than 4.9 mg/kg. Each clinical picture was much worse when the arsenic intake was large. The only exception is the pigmentation in which both groups exhibited the identical percentage. The fact that the pigmentation did not show a parallel relation to the amount of arsenic intake, like the other clinical pictures, should be viewed seriously. Taking into consideration also the fact that there are some serious cases with no pigmentation, it is not appropriate to judge the seriousness of arsenic poisoning from the pigmentation condition only. It is not yet known how this pigmentation arises, but its relation to adrenals can be practically considered and we did perform some studies relating to the adrenals (to be mentioned later).

2) Relation Between Amount of Arsenic in the Blood and Clinical Symptoms

There has been no report on the amount of arsenic in the blood of a healthy infant. Hence, it was difficult to judge what was normal in regard to the amount of arsenic in the infants affected with arsenic poisoning. As a reference, arsenic concentration in blood of a healthy adult is shown in Table 4. For infants taking ordinary food as well as adults, the amount of arsenic in the blood is naturally expected to be affected by the amount of arsenic in food or drinks taken. For example, by taking seaweeds (1-1.2 γ/gm Williams [12]) or small shrimps (130 γ/gm Chapman [13]) which contain a fairly large quantity of arsenic (generally, fish contains a relatively large quantity of arsenic), the normal range of arsenic concentration in the blood will

TABLE 3. RELATIONSHIP BETWEEN TOTAL AMOUNT OF As INTAKE AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Clinical Symptoms \ Total Amount of As Per kg		10mg/kg< 7 cases	4.9mg/kg> 7 cases
Digestive Symptoms (Diarrhea, Vomitus, Anorexia)		7 (100%)	4 (57%)
Respiratory Symptoms (Cough, Hoarseness)		7 (100)	3 (43)
Fever		7 (100)	4 (57)
Liver Enlargement 4cm<		3 (43)	0 (0)
Spleen Enlargement		2 (29)	1 (14)
Skin Symptoms	Exanthema	6 (86)	3 (43)
	Pigmentation +4<	4 (57)	4 (57)
	Desquamation +4<	5 (71)	3 (43)
	Loss of Hair	2 (29)	1 (14)
	Nails Change	6 (86)	5 (71)
Blood Picture	Hb (80%>)	3 (43)	1 (14)
	R. B. C. (400×10^4>)	3 (43)	2 (29)
	W. B. C. (7000>)	3 (43)	1 (14)
	Neutrophilic W. B. C. (20%>)	5 (71)	4 (57)
	Eosinophilic W. B. C. (4%<)	3 (43)	5 (71)
Liver Function	Cob. R. (>3. 4. 5<)	4 (57)	4 (57)
	Cad. R. (>6. 7. 8<)	3 (43)	3 (43)
	Bromsulfalein Test	4 (57)	1 (14)
Thorn's Test		1 (14. 0)	0 (0)
Changes of E. C. G		0 (0)	0 (0)

TABLE 4. AMOUNT OF As IN BLOOD OF NORMAL ADULT

Amount of As	Author
0. 837/g	Billeter, O. et al [9]
0. 947/g	Kingsley, G. R. et al [10]
0 ~ 0. 227/g	Iwataki, N. [11]

become high. However, the average infants' food is almost all fixed, except for infants in the weaning period. For the pregnant woman, an arsenic concentration in the blood is high, reaching a maximum of 1.44 γ/gm (5-6th month of pregnancy). Consequently, arsenic concentration in the blood of new born infants could be considered high, but there is no literature on its measurement. For the above reasons, the amount of arsenic in the blood of healthy infants was measured as a contrast. It was 0-0.3 γ/gm. As the arsenic concentration in blood for the infants with arsenic poisoning measured 6 months later is in the range of approximately 0-0.3 γ/gm, the arsenic concentration in the normal blood of infants can be considered to be about this level.

An arsenic concentration in the blood of infants with arsenic poisoning this time was, as announced in the first report, almost all above 0.3 γ/gm, reaching a maximum of 3.1 γ/gm. The comparisons of arsenic concentration in blood and the clinical picture are shown in Tables 5 and 6. The clinical pictures in these two tables are those observed at the first consultation, and those observed from each arsenic determination in the blood, respectively. It is clear from this table that respiratory symptoms, fever, liver enlargment, anemia, and the rate of reduction of white blood cells and neutrophilic white blood cells are high in the group with a high arsenic concentration in the blood, and a clear difference can be seen in the bromsulfalein test and Thorn test*. Contrary to this, pigmentation, desquamation, and nails, change are not related to an arsenic concentration in the blood. Table 6 shows a comparison between the amount of arsenic in the blood and the clinical picture during an arsenic determination. This is different from the previous table in that a clear difference appeared in the digestive symptoms and B.S.P. but, on the other hand, no difference was detected in liver enlargment and the blood picture. The fact that a conspicuous difference was seen in digestive symptoms in the latter is though to be due to the effect of arsenic discharge to the intestinal canal. The symptoms of liver enlargement and B.S.P. point to the influence of arsenic on the liver. Also, from the adrenal cortex function

*Translator's note: This is a test of adrenal cortical response after injection of ACTH or of epinephrine.

TABLE 5. REPLATIONSHIP BETWEEN AMOUNT OF As IN BLOOD AND CLINICAL SYMPTOMS AT EACH DETERMINATION

Clinical Symptomes \ Amount of As in Blood		1.1γ/cc< 14 cases	1.0γ/cc> 16 cases
Digestive Symptoms		8 (57%)	2 (13%)
Respiratory symptoms		7 (50%)	2 (13%)
Fever		3 (21%)	1 (6%)
Liver Enlargement		8 (57%)	8 (50%)
Spleen Enlargement		2 (14%)	1 (6%)
Skin Symptoms	Exanthema	2 (14%)	4 (25%)
	Pigmentation	8 (57%)	10 (62%)
	Desquamation	5 (36%)	6 (37%)
	Loss of Hair	1 (7%)	2 (13%)
	Nails Change	8 (57%)	8 (50%)
Blood Picture	Hb (80%>)	3 (21%)	2 (13%)
	R.B.C. (400×10⁴>)	5 (36%)	4 (25%)
	W.B.C. (7000>)	3 (21%)	4 (25%)
	Neutrophilic W.B.C. (20%>)	4 (29%)	2 (13%)
	Eosinophilic W.B.C. (4%<)	7 (50%)	8 (50%)
Liver Function	Coh.R. >2.4.5<	5 (36%)	6 (37%)
	Cad.R. >6.7.8<	2 (14%)	3 (19%)
	Bromsulfalein Test	7 (50%)	3 (19%)
Thorn's Test		5/12 (42%)	0/5 0%
Changes of E.C.G.		1/11 (9%)	2/11 (18%)

test and Thorn test, arsenic seemed to infringe on the adrenals in some way, but no difference in pigmentation was seen. Taking into consideration also the case in which the pigmentation was not seen in serious arsenic poisoning, the relationship between the adrenals and arsenmelanosis during arsenic poisoning is difficult to explain simply.

3) Relation Between Amount of Arsenic in Urine and Feces and Clinical Symptoms

The normal amount of arsenic in the urine and feces holds also for the blood. That is, it is frequently influenced by food; also, data on infants are not available. As a reference, the normal values for adults are shown in Table 7. If various reports are summarized, approximately 0-850 γ/1 for urine and 0-80 γ/100 gm dry weight for feces become the normal range. However, for infants, our measurement data showed the normal range to be 0-50 γ/1 for urine and 0-5 γ/10 gm dry weight for feces (For the amount of arsenic in the urine and feces in infants affected with arsenic poisoning, please refer to Figure 10 in the first report). The comparisons between the clinical symptoms and the amount of arsenic in the urine and feces are shown in Tables 8 and 9. Nineteen cases were observed. In early September, the amount of arsenic in the urine was 40-590 γ/1, and that in the feces was 30-2 γ/10 gm.

i) Comparison between amount of arsenic in the urine and clinical symptoms

When nineteen cases were divided into one group of 10 cases with the amount of arsenic above 20 γ/dl and the other group of 9 cases with the amount of arsenic below 20 γ/dl and a comparison of symptoms at the first consultation for both groups was made, the only difference seen was in the Hb (The former had 6 cases with Hb below 80%, while the latter had none). Next, the clinical symptoms at the time of arsenic determination in the urine are compared and shown in Table 8.

Digestive and respiratory symptoms, nail changes, and reduction in neutrophilic white blood cells are seen in the group with a large amount of arsenic discharge. On the other hand, liver enlargement and pigmentation are more frequently seen in the group with a small amount of arsenic discharge. The reasons for the small amount of discharge into the urine may be the following: 1) the intake is small, 2) liberation of arsenic from the tissues is poor, and 3) the discharge is bad due to kidney trouble. In any event, in the group with a large arsenic discharge in the urine, liver enlargement is

rare, liver function trouble (cobalt and cadmium reaction) is frequent, and difficulties in the adrenal function (Thorn's test) are rare. In the group with a small amount of arsenic discharge in the urine, liver enlargement is frequent, liver function trouble is rare, and adrenal trouble is frequent. This shows that there is not much relation between liver enlargement and liver function. Even if an impediment is exhibited in serum colloid reaction, an arsenic discharge into the urine is promoted if liver enlargement is milder and the adrenal function is closer to normal.

ii) Amount of arsenic in feces and clinical symptoms

The symptoms at the first consultation are compared in Table 9. As is seen from this table, the digestive symptoms were stronger when the amount of discharge was rather small. Kesser and others [5] stated that the continuous oral administration of arsenic results in the lowering of arsenic absorption from the intestinal wall and in an increase in the amount of arsenic in the feces. According to this report, when the amount of arsenic in the feces is small, the intestinal wall has difficulty adjusting; thus, the digestive symptoms, liver enlargement, and liver function (B.S.P.) trouble are common.

4) Relation Between Amount of Arsenic Intake and Discharge and Clinical Symptoms

The cases in which the amount of arsenic intake was relatively accurately determined and the amount of arsenic in the blood, urine, and feces was measured early were rare. However, it is clear that these are the most important data for clarifying the true nature of chronic arsenic poisoning. Even though there were only 5 cases, we show these very important data we have collected in Table 10. The fact that the fifth case with a large total amount of arsenic intake and a small amount of arsenic discharge shows the worst clinical symptoms seems to be a natural result of the true nature of poisoning.

5) Comparison of Amount of Arsenic in Hair and Clinical Symptoms

The clinical symptoms were those at the first consultation. Again, the amount of arsenic in the normal hair of infants is not reported. The normal values for adults are as shown in Table 11. Our study indicated the amount of arsenic in the hair of normal infants to be 0-50 γ/100 gm. In most cases (22 out of 24 cases), the amount of arsenic contained was more than 1 mg/100 gm, with a maximum reaching 10 mg/100 gm. This is a quite high concentration compared to the previous arsenic poisoning reports. (2.2 mg/100 gm Heffter; 0.06-1.3 mg/100 gm Kölsch).

TABLE 6. RELATIONSHIP BETWEEN AMOUNT OF As IN BLOOD AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Amount of As in Blood / Clinical Symptoms		2.1γ/cc< (8 cases)		2.0~1.1γ/cc (5 cases)		1.0γ/cc> (9 cases)	
Digestive Symptoms		6	(75%)	5	(100%)	6	(67%)
Respiratory Symptoms		6	(75%)	3	(60%)	2	(22%)
Fever		6	(75%)	3	(60%)	3	(33%)
Liver Enlargement		6	(75%)	2	(40%)	3	(33%)
Spleen Enlargement		2	(25%)	0	(0%)	0	(0%)
Skin Symptoms	Exantheme	7	(87%)	4	(80%)	6	(67)
	Pigmentation	4	(50%)	3	(60%)	6	(67%)
	Desquamation	4	(50%)	2	(40%)	4	(44%)
	Loss of Hair	2	(25%)	0	(0%)	2	(22%)
	Nails Change	6	(75%)	4	(80%)	2	(22%)
Blood Picture	Hb (80%>)	5	(63%)	3	(60%)	3	(33%)
	R.B.C. (400×10⁴>)	6	(75%)	3	(60%)	2	(22%)
	W.B.C. (7000>)	4	(50%)	4	(80%)	2	(22%)
	Neutrophilic W.B.C (20%>)	7	(87%)	4	(80%)	3	(33%)
	Eosinophilic W.B.C (4%<)	4	(50%)	2	(40%)	2	(22%)
Liver Function	Cob.R. >3.4.5<	3	(38%)	2	(40%)	4	(44%)
	Cad.R. >6.7.8<	2	(25%)	0	(0%)	1	(11%)
	Bromsulfalein Test	4	(50%)	2	(40%)	3	(33%)
Thorn's Test		4	(50%)	3	(60%)	%	(0%)
Changes of E.C.G		1	(13%)	0	(0%)	%	(0%)

TABLE 7. AMOUNT OF As IN URINE OF NORMAL ADULT

Urine	Feces	Author
830γ/l>	80γ/100g>	Schwarz. L.[6]
0~850γ/day		Mattice M.R.et al[14]
0~690γ/l		Mayers. M. R.[15]
106γ/l		Kingsley.G.R.et al[16]
130γ/l		Pinto
12~834γ/l average 141γ/l	Ca. 40γ/100g	Iwataki, N.[11]
	0~20γ/10g dry weight	Neal, K.[17]
100γ/l>		Künkele[18]
0~850γ/l		Johnstone. R. T.[19]
0~650γ/l		Bang. I.[20]
13~130γ/l		Tate. M.[21]
50~100γ/l		Koelsch. F.[22]

As shown in Table 12, the greater the amount of arsenic in the hair, the more serious the clinical symptoms become, indicating that the degree of symptoms is proportional to the amount of arsenic. However, the symptoms with more than 4% eosinophilic white blood cells are frequent in the group with a small amount of arsenic in the hair. An individual with an increase in eosinophilic white blood cells is generally considered to have a favorable prognosis. It is doubtful whether an increase in eosinophilic white blood cells can be related to adrenal and pituitary bodies.

A conspicuous parallel relationship between the clinical symptoms and the amount of arsenic in the hair can be said to indicate tissue deposition as an accurate index of arsenic intake and discharge. Further, it was extremely convenient not having to cut the hair of infants often, unlike adults.

6) Amount of Arsenic Contained in Blister Fluid Obtained by Pasting a Blister-Blaster on the Skin and Clinical Symptoms

The clinical symptoms are those at the first consultation. Like hair, this was considered to be a good index of clinical symptoms. As shown in Table 13, the amount of arsenic in blister fluid indicated a high concentration in many cases, but its concentration and the level of symptoms did not necessarily go along in parallel. This may be explained by the fact that arsenic in the skin, unlike that in hair, is relatively mobile, or that, because of the strong discharge of arsenic due to perspiration during the summer period, a parallel relation with the clinical symptoms was not recognizable.

TABLE 8. RELATIONSHIP BETWEEN AMOUNT OF As IN URINE OF PATIENTS AND CLINICAL SYMPTOMS AT EACH DETERMINATION

Amounte of As in Urine / Clinical Symptoms		31γ/dl< 6 cases		30~16γ/dl 7 cases		15γ/dl> 6 cases	
Digestive Symptoms		4	(67%)	5	(71%)	3	(50%)
Respiratory Symptoms		4	(67)	2	(29)	1	(17)
Fever		2	(33)	1	(14)	2	(33)
Liver Enlargement		1	(17)	5	(71)	4	(67)
Spleen Enlargement		0	(0)	2	(29)	0	(0)
Skin Symptoms	Exanthema	1	(17)	2	(29)	1	(17)
	Pigmentation	1	(17)	5	(71)	5	(83)
	Desquamation	1	(17)	3	(43)	2	(33)
	Loss of Hair	1	(17)	2	(29)	2	(33)
	Nails Change	5	(83)	2	(29)	3	(50)
Blood Picture	Hb. (80%>)	0	(0)	0	(0)	1	(17)
	R. B. C. (400×10⁴>)	2	(33)	1	(14)	1	(17)
	W. B. C. (7000>)	1	(17)	1	(14)	1	(17)
	Neutrophilic W. B. C. (20%>)	3	(50)	0	(0)	1	(17)
	Eosinophilic W. B. C. (4%<)	4	(67)	3	(43)	4	(67)
Liver Function	Cob. R. >3. 4. 5<	3	(50)	4	(57)	1	(17)
	Cad. R. >6. 7. 8<	3	(50)	2	(29)	0	(0)
	Bromsulfalein Test	3	(50)	3	(43)	4	(67)
Thorn's Test		2	(33)	2	(29)	3	(50)
Changes of E. C. G.		1	(17)	0	(0)	0	(0)

7) Amount of Arsenic Contained in Nails and Clinical Symptoms

The amount of arsenic in normal adults is roughly 17.2 γ/100 gm [6], and our study showed 2.5 γ/100 gm for healthy adults. In the case of arsenic poisoning, there are reports of 0.35 mg/100 gm or 2-13 mg/100 gm (Heffter [5]). Among our cases, the maximum was 30 mg/100 gm (3 γ/10 mg). There was one case of a zero amount of arsenic, but the nails are assumed to be those before arsenic intake. If the portion of Mees' band is taken, an extremely high value results. Thus, the amount of arsenic contained in the nails is greatly affected by a time relation.

Table 14 compares 12 cases above 1.0 γ/10 mg and 12 cases below 0.9 γ/10 mg with the clinical pictures. Other than the blood picture, no parallel relations can be seen. This again seems to be due to the time lag of the nails collected.

IV. RELATION BETWEEN SERUM PROTEIN FRACTION AND CLINICAL SYMPTOMS

The serum protein fraction of arsenic poisoning patients has already been discussed in the first report. In it, a reduction in albumin and an increase in β and γ globulin were reported. This was also confirmed in the acute and chronic arsenic poisoning of rabbits. This can be considered as one symptom of reduced adrenal function, based on Lewis' [26] report in which serum albumin decreased and γ globulin increased during adrenal malfunctioning. Table 15 compares this serum protein fraction with the clinical symptoms. The correlation between the clinical picture and the change in protein fraction, expecially the fluctuation in albumin and β and γ globulin, can not be observed. Rather, one of the points which should be exphasized is that the serum protein fraction was approximately normal in the case of serious symptoms with a liver enlargement of 8 cm. Therefore, the relation between the internal organs producing serum proteins and the arsenic poisoning can be presumed to be complex.

V. RELATION BETWEEN SERUM SH GROUP AND CLINICAL SYMPTOMS

The determination of the serum SH group is done by Hata's electric titration method. The relation between the change in serum SH of in-patients and the treatment is shown in Figure 1. The low values are shown at first, but

TABLE 9. RELATIONSHIP BETWEEN AMOUNT OF As IN FECES AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Clinical Symptoms \ Amount of As in Feces		15γ/g< (6 cases)	14~9γ/g (9 cases)	8γ/g> (4 cases)
Digestive Symptoms		3 (50%)	8 (89%)	4 (100%)
Respiratory Symptoms		3 (50)	5 (56)	2 (50)
Fever		5 (83)	5 (56)	1 (25)
Liver Enlargement		3 (50)	5 (56)	3 (75)
Spleen Enlargement		1 (17)	1 (11)	0 (0)
Skin Symptoms	Exanthema	4 (67)	8 (89)	3 (75)
	Pigmentation	4 (47)	5 (56)	2 (50)
	Desquamation	4 (67)	3 (33)	0 (0)
	Loss of Hair	3 (50)	0 (0)	1 (25)
	Nails Change	4 (67)	5 (56)	3 (75)
Blood Picture	Hb. (80%>)	3 (50)	6 (67)	2 (50)
	R. B. C. (400×10⁴>)	3 (50)	5 (56)	3 (75)
	W. B. C. (70000>)	3 (50)	5 (56)	2 (50)
	Neutrophilic W. B. C. (20%>)	6 (100)	7 (78)	3 (75)
	Eosinophilic W. B. C. (4%<)	3 (50)	3 (33)	1 (25)
Liver Function	Cob. R. >3.4 5<	2 (33)	4 (44)	2 (50)
	Cad. R. >6.7.8<	2 (33)	2 (22)	1 (25)
	Bromsulfalein Test	3 (50)	7 (78)	1 (25)
Thorn's Test		3 (50)	3 (33)	2 (50)
Changes of E. C. G.		0 (0)	1 (11)	0 (0)

gradually the normal or the high values are attained. It is clear that arsenic attacks SH group of tissue protein, forming a metal-protein complex, and causes poisoning symptoms. In general, the SH group can be classified into the soluble SH groups, mostly glutathione, and the fixed SH groups which combine with tissue protein. Further, Barron [27] classified the fixed SH groups according to their reaction as follows: marked, sluggish, and freely reactive. Regardless of with which SH group arsenic reacts, it can be concluded that arsenic combines with SH groups and causes a reduction in the serum SH group.

TABLE 10. RELATIONSHIP BETWEEN AMOUNT OF As AND CLINICAL AND LABORATORY FINDINGS IN PATIENTS

		1	2	3	4	5
As-intake	Total Amount	54.4mg	79.8	74.4	80	216.0
	Total Amouut per kg	10.0mg	10.2	10.6	15.6	29.2
	Total Amount per day per kg	0.63mg	0.49	0.62	0.34	0.36
Digestive Symptoms		+	−	−	+	+
Respiratory Symptoms		−	−	−	+	+
Fever		+	−	−	−	+
Skin Symptoms	Exanthema	−	+	+	+	+
	Pigmentation	−	++	+	++	+++
	Desquamation	−	+++	−	−	−
	Loss of Hair	−	−		−	
	Nails Change	−	+	−	−	
Liver Enlargement		3 (cm)	2.0	2.0	4.0	8.0
Spleen Enlargement		0 (cm)	0	0.5	0	2.0
Liver Function	Cob. R.	R_1	R_3	R_1	R_3	R_7
	Cad. R.	R_6	R_6	R_6	R_7	R_6
	Bromsulfalein Test	0 (%)	0	10	5	20
Blood Picture	Hb.	90 (%)	73	95	75	41
	R. B. C.	421 ($\times 10^4$)	387	475	275	210
	W. B. C.	7100	7200	9000	5600	2900
	Neutrophilic W. B. C.	13.2 (%)	10.0	9.2	16.0	14.0
	Eosinophilic W. B. C.	3.2 (%)	2.0	5.2	0.8	1.0
Function of Adrenal cortex	Thorn's Test	−	−	+		+
	Serum Na/K	126mEq/l 6.7mqE/l	120 4.6	124 5.1	125 5.6	129 6.0
	Urine 17KS	0.11 mg/l	1.24	0	0	0.91
Serum-SH			0.41	0.50	0.47	0.42
Al/Gl Ratio in Serum		1.28	1.29	1.29	0.82	1.42
As-Content	Blood	2.6 γ/cc	2.2	1.9	0.6	2.2
	Hair	34 γ/gm	34	50	70	60
	Nail			1.4γ/10mg	0.03	
	Urine	5.0γ/dl	32.0	19.0	46.5	17.0
	Feces	1.1γ/g dry weight	0.2	0.8	1.0	0.9
	Blister Fluid		12.0γ/cc			2.0

TABLE 11. AMOUNT OF As IN HAIR OF NORMAL ADULT

Amount of As γ/100gm	Author
27~77	Seip, ö. [23]
60~138	Kölsch, F. [24]
300	Künkele, F. [18]
30	Johnstone. R. T. [23]
150~200	Iwataki, N. [11]
9.7	Biol,Dat. Kinderarzt [6]
0~30	Heffter, A. [5]

TABLE 12. RELATIONSHIP BETWEEN AMOUNT OF As IN HAIR AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Clinical Symptoms \ Amount of As in Hair		41γ/gm 9 cases		40~26γ/gm 9 cases		25γ/gm 7 cases	
Digestive Symptoms		8	(89%)	6	(67%)	6	(86%)
Respiratory Symptoms		5	(56%)	5	(56%)	1	(14%)
Fever		3	(33%)	6	(67%)	1	(14%)
Liver Enlargement		6	(67%)	5	(56%)	4	(57%)
Spleen Enlargment		1	(11%)	1	(11%)	0	(0%)
Skin Symptoms	Exanthema	6	(67%)	8	(89%)	3	(43%)
	Pigmentation	7	(78%)	4	(45%)	3	(43%)
	Desquamation	4	(45%)	3	(33%)	2	(28%)
	Loss of Hair	2	(22%)	1	(11%)	0	(0%)
	Nails Change	5	(56%)	6	(67%)	5	(71%)
Blood Picture	Hb. (80%>)	5	(56%)	2	(22%)	1	(14%)
	R. B. C. (400×10^4>)	6	(67%)	3	(33%)	3	(43%)
	W. B. C. (7000>)	7	(78%)	6	(67%)	0	(0%)
	Neutrophilic W. B. C. (20%>)	8	(89%)	7	(78%)	3	(43%)
	Eosinophilic W. B. C. (4%<)	0	(0%)	7	(78%)	4	(57%)
Liver Function	Cob. R. >3. 4. 5<	4	(45%)	4	(45%)	3	(43%)
	Cad. R. >6. 7. 8<	5	(56%)	3	(33%)	3	(43%)
	Bromsulfalein Test	5	(56%)	5	(56%)	1	(14%)
Thorn's Test		4	(45%)	4	(45%)	0	(0%)
Changes of E. C. G.		1	(11%)	1	(11%)	1	(14%)

It is also clear the with the administration of various drugs, arsenic is freed from a metal-protein complex in tissues, and tissues and serum SH are restored to the original condition. Shacter and others [28] recognized a fall in plasma SH by adrenalectomy and reported that an administration of cortisone and corticotropin would prevent it. This fact indicates a close relation between adrenal cortex function and serum SH level. Table 16 compares the clinical pictures at the first consultation between 5 cases in which the amount of serum SH is 0.46-0.41, and 6 cases in which the amount of serum SH is within what is considered a normal range, 0.47-0.53. In a group with a low serum SH, the changes in fever, pigmentation, desquamation, nails, blood picture, and Thorn test are clearly fewer. The following can be considered as an explanation for this fact. When the serum SH concerning acute or chronic arsenic poisoning of rabbits is measured, the plasma-SH level goes down a few minutes after the administration of arsenic, but recovers to the original level approximately 2 weeks after the termination of arsenic administration. Two meanings can be attached to this fluctuation in the SH level: 1) based on adrenal extraction and tissue impediment mentioned by Shacter and others, a lowering in plasma-SH level is an impediment to the supply source of plasma-SH and 2) plasma-SH possesses a protective function in preventing the toxic action to SH enzymes which are important to the vital phenomena within tissues.

From this fact, a lowering of the plasma-SH level is, on the one hand, a demonstration of a defensive force; on the other hand, it signifies a dangerous condition as an indication of tissue impediment. Consequently, we like to think that the lowering of the plasma-SH level in a short time after arsenic administration is a case of 2), while a lowering of plasma-SH over a long period of time and even after the termination of arsenic administration is a case of 1).

The fact that the plasma-SH values are low in the group with a relatively good clinical condition can be regarded as a reserve defensive force in serum to prevent the arsenic invasion into tissues. Although it will be mentioned in the section on 17 K.S., 3 out of 5 cases showing the low -SH values exhibited

TABLE 13. RELATIONSHIP BETWEEN THE AMOUNT OF As IN VESICATED BLISTER FLUID IN THE PATIENTS AND THEIR CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Clinical Symptoms \ Amount of As in Blister Fluid		6.1γ/cc< 6 cases		6.0~2.1γ/cc 5 cases		2.0γ/cc> 6 cases	
Digestive Symptoms		4	(67%)	4	(80%)	5	(83%)
Respiratory Symptoms		5	(83)	2	(40)	1	(17)
Fever		4	(67)	1	(20)	2	(33)
Liver Enlargement		3	(50)	1	(20)	5	(83)
Spleen Enlargement		0	(0)	1	(20)	1	(17)
Skin Symptoms	Exanthema	4	(67)	2	(40)	4	(67)
	Pigmentation	3	(50)	2	(20)	4	(67)
	Desquamation	3	(50)	2	(20)	3	(50)
	Loss of Hair	2	(33)	0		0	
	Nails Change	4	(67)	4	(80)	2	(33)
Blood Picture	Hb. (80%>)	1	(17)	1	(20)	1	(17)
	R.B.C. (400×10⁴>)	3	(50)	2	(40)	1	(17)
	W.B.C. (7000>)	3	(50)	1	(20)	1	(17)
	Neutrophilic W.B.C. (20%>)	3	(50)	2	(40)	1	(17)
	Eosinophilic W.B.C. (4%<)	2	(33)	3	(60)	2	(33)
Liver Function	Cob.R. >3.4.5>	1	(17)	3	(60)	6	(100)
	Cad.R. >6.7.8<	0		0		0	
	Bromsufalein Test	4	(67)	1	(20)	4	(67)
Thorn's Test		2	(33)	1	(20)	1	(17)
Changes of E.C.G		0		0		0	

high 17 K.S. values (0.91; 1.24; 2.28 mg/l). Further, if we realize that only 6 out of 20 patients indicated a positive 17 K.S. upon hospitalization, then in these cases, a reduction of adrenal function is small, and thus a lowering of SH due to the adrenal influence should not occur. It is dangerous to diagnose the disease condition by the lowering of plasma-SH during arsenic poisoning. It should be noted in this regard that Shoenback's [29] paper reported that a lowering of plasma-SH was seen during various diseases, but no special correlation was detected.

TABLE 14. RELATIONSHIP BETWEEN AMOUNT OF As IN NAILS AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Clinical Symptoms \ Amount of As in Nails		1.07/10mg< 12cases	0.97/10mg> 12cases
Digestive Symptoms		10 (83%)	11 (92%)
Respiratory Symptoms		6 (50%)	7 (58%)
Fever		8 (67%)	6 (50%)
Liver Enlargment		7 (58%)	4 (33%)
Spleen Enlargement		1 (8%)	0 (0%)
Skin Symptoms	Exanthema	7 (58%)	10 (83%)
	Pigmentation	8 (67%)	9 (75%)
	Desquamation	2 (17%)	6 (50%)
	Loss of Hair	4/11 (36%)	1/10 (10%)
	Nails Change	10 (83%)	10 (83%)
Blood Picture	Hb (80%>)	0 (0%)	6 (50%)
	R.B.C. (400×10^4>)	1 (8%)	7 (58%)
	W.B.C. (7000>)	3 (25%)	5 (42%)
	Neutrophilic W.B.C. (20%>)	5 (42%)	11 (92%)
	Eosinophilic W.B.C. (4%<)	6 (50%)	7 (58%)
Liver Function	Coh.R >3.4.5<	6 (50%)	5 (42%)
	Cad.R >6.7.8<	1 (8%)	4 (33%)
	Bromsulfalein Test	5/6 (83%)	2/10 (20%)
Thorn's Test		3/6 (50%)	2/8 (25%)
Changes of E.C.G.		0/10 (0%)	2/11 (18%)

VI. CONCENTRATION OF Na AND K IN SERUM AND CLINICAL SYMPTOMS

Na and K in serum were measured by a flame photometer. As shown in Table 17, a high K and a low Na were displayed. This trend was restored to the nearly normal range in the second measurement one month later, but 2 out of 10 cases showed a high Na value, and 3 out of 10 cases showed a high K value. It is expected that these would return to the normal range as the clinical symptoms are improved. In the first measurement, only 3 cases showed abnormal

TABLE 15. RELATIONSHIP BETWEEN SERUM PROTEIN FRACTION AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

	Protein concentratin	Albumin	Globulin				A/G	Digestive Symptoms	Respiratory Symptoms	Fever	Exanthema	Pigmentation	Desquamation	Loss of Hair	Nails Change	Liver Enlargement	Spleen Enlargement	Hb (%)	R.B.C. ×10⁴	W.B.C. ×10³	Neutrophilic W.B.C. (%)	Eosinophilic W.B.C.	Cob. R.	Cad. R.	Bromsulfalein Test	Thorn's Test	Changes of E.C.G.
			α1	α2	β	γ																					
	(7.0%)	(59.8%)	(4.7)	(11.2)	(9.4)	(15.0)	(1.48)																				
1	7.3	55.6	3.0	11.9	12.3	17.2	1.25	++	±	+	+	+++	++	−	+	5cm	2cm	80	410	78	19	7	3	6	−	−	−
2	6.9	63.9	2.1	11.3	13.2	9.5	1.72	±	−	−	+	+	+	−	−	4	−	83	414	140	34	8	1	6	+	−	−
3	7.8	52.2	4.2	13.2	14.2	16.2	1.09	+	−	±	±	++	−	−	−	1	−	100	507	71	51	1	7	6	−	−	−
4	7.7	56.4	5.8	11.7	8.7	17.4	1.29	+	+	+	+	+	+	−	+	3	−	73	385	50	20	5	3	6	−	−	−
5	6.2	56.2	4.7	15.5	14.0	9.6	1.28	++	+	++	−	−	−	±	+	3	−	90	421	71	13	3	1	9	−	−	−
6	7.2	56.5	4.2	11.4	14.8	13.1	1.29	+	+	−	−	+	+	−	+	5	−	80	336	57	4	3	2	7	−	+	−
7	7.2	50.8	6.0	10.9	14.7	17.6	1.03	+	+	±	+	++	++	+	+	4	−	90	392	66	6	1	5	8	+	+	−
8	7.0	45.1	4.9	17.0	14.2	18.8	0.82	++	+	±	+	++	−	−	+	4	−	75	275	56	16	1	3	7	+	−	−
9	7.2	60.5	3.3	6.6	11.1	18.5	1.53	+	−	−	−	−		±	−	3	−	78	505	96	14	1	1	6	+	−	−
10	9.1	54.6	5.2	10.5	12.5	17.2	1.20	++	++	++	+	+++	+++	−	+	3	−	80	378	68	17	11	3	6	+	+	−
11	5.8	58.8	5.9	8.1	9.5	17.7	1.42	+	+	++	++	+	++	−	+	8	2	41	210	29	14	1	10	4	+	−	±

values for both Na and K. According to Table 18 which compared the first serum K value and the symptoms at the first consultation, strong symptoms were observed in the cases with medium high values of K. It is interesting to note that a case in the group with K concentration in serum of more than 5.6 mEq/l shows a change in the electrocardiogram. As the electrocardiogram observation is discussed in detail in the report on the clinical findings, it is omitted here. As seen in Table 19, the relation between Na concentration in the serum and the clinical symptoms is not conspicuous. In regard to the comparison between the clinical symptoms and the three cases in which both Na and K showed abnormal values, it is difficult to state its relation because of a small sample. The question arises as to whether an increase in K or a decrease in Na in the serum is a condition during arsenic poisoning or during the recovery period. If we refer to the experimental data on chronic arsenic poisoning of rabbits, which we are planning to report on next time, the K in the serum rises after the arsenic administration and maintains a high value for several weeks following the termination of arsenic administration. Na initially shows a low value, but recovers to the original value several weeks after the termination of arsenic administration. That is, during arsenic poisoning, K in the serum tends to rise, and Na tends to decrease. Consequently, the change in the patient's heart which appeared in the electrocardiogram is not due to hypopotassemia. In any event, the hypopotassemia and electrocardiogram is not due to hypopotassemia. In any event, the hypopotassemia and electrocardiogram have recently become a target of debate. The lowering of ST, the lowering and inversion of T and an elevation in U may be considered to be characteristics of pure hypopotassemia [30], but there are opposing opinions, taking into consideration such things as K in the cell, the ratio of K inside and outside of the cell, and K in the myocardium. On the relation between hyperpotassemia and the electrocardiogram, Winkler [31], Lenzi [32], and others have reported an elevation in T, a drop in ST, a prolongation of the depolarisation time, and a disappearance of P, depending on its level. Whether hyperpotassemia in arsenic poisoning and the change in the electrocardiogram can be directly related or not is questionable. In our experience with chronic arsenic poisoning of rabbits, its correlation was not recognized.

Consequently, it may be that this hyperpotassemia is related to adrenal function or kidney malfunction only, and not to the electrocardiogram.

The concentrations of Na and K in serum are affected not only by the adrenal function, but also by the condition of the ureter or digestive tract. When there was a change in the ureter in arsenic poisoning, almost no change in the urine was found in our cases. It can be assumed that reabsorption of Na and K is impeded, and they are discharged into the urine, thereby causing a decrease of Na and K in blood. Also if diarrhea is violent, a loss of K occurs. By the way, among the infants affected with arsenic poisoning, a low Na symptom as mentioned previously exists, but K shows the high value. Ultimately, its cause must probably be traced back to the adrenals.

VII. 17 K.S. IN URINE AND CLINICAL SYMPTOMS

The measurement was performed on 20 in-patients. The results are shown in Figure 2. In early September, 14 cases showed 0 mg/l, and the other 6 cases showed 0.11, 0.60, 0.62, 0.91, 1.24, 2.28 mg/l each. For infants, 17 KS in urine of around 0 mg/l is normal [33], and whether this is normal or not for the patients can not be determined. If we refer to the results of rabbit experiments, the lowering of 17 KS in urine in arsenic poisoning was confirmed. Consequently, in the case of affected infants also, the first measurement data were assumed to relate to low adrenal function. Further, the fact that the amount of 17 KS in the urine increased with time was regarded as a hyperactive condition. The comparisons of the clinical pictures at the first consultation between 13 out of 14 cases which showed a decreasing amount of 17 KS in the urine, and 6 cases which did not are shown in Table 20. In this table, large differences in liver enlargement and pigmentation were observed between the two groups. Especially in regard to pigmentation, 2 cases belonging to the group in which 17 KS existed had a very small amount of 17 KS. No relation to the Thorn test was recognized.

VIII. ARSENIC POISONING AND ADRENAL FUNCTION

Of the tests which have been mentioned, Thorn's and Long's tests will be discussed in the report on the clinical findings. Looking at the behavior of the plasma-SH, Na and K in serum, and 17 KS in feces* from an adrenal function view point, this indicates a condition of depressed adrenal function during the relatively initial period of poisoning. This suggests that some symptoms of subacute or chronic arsenic poisoning are caused by the lowering of the adrenal function due to arsenic poisoning. Here, the natural question arose as to whether the pigmentation during arsenic poisoning was due to the same mechanism as that of Addison's disease. It has already been proven that the pigment which is deposited during arsenic poisoning is melanin. It is also known that a strong histopathological change occurs in the adrenal body, expecially in the adrenal cortex. Furthermore, Ujiie [34] reported that the anterior pituitary is related to arsenic discharge, indicating a relation between arsenic poisoning and the pituitary body. It seems appropriate to estimate from the pigmentation mechanism of Addison's disease that of arsenic poisoning, and we shall touch on it briefly below.

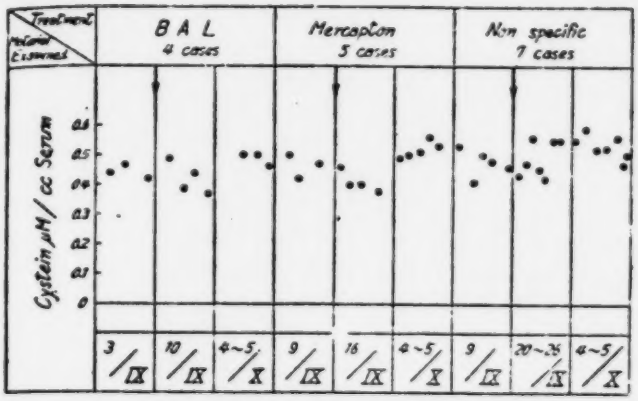

Figure 1. Changes of amount of SH in plasma of in-patients.

The tyrosin-tyrosinase reaction to form melanin takes place within the melanocyte on the epidermal-dermal junction [35]. When the function of this cell is accelerated, pigmentation is generated, and when it is destroyed, the pigment escapes and leukoderma occurs. The melanocyte generates pigments with the help of tyrosinase in this cell. This enzyme requires copper for its activity. Consequently, if the -SH compound which combines with copper is given, the melanin formation is inhibited. Also if

TABLE 16. RELATIONSHIP BETWEEN AMOUNT OF -SH IN PLASMA AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Clinical Symptoms \ Amount of -SH in Plasma cystein μM/cc		0.47~0.53 Normal 6 cases	0.46 > 5 cases
Digestive Symptoms		4 (67%)	4 (80%)
Respiratory Symptoms		4 (67)	3 (60)
Fever		4 (67)	2 (40)
Liver Enlargement		4 (67)	4 (80)
Spleen Enlargement		1 (17)	1 (20)
Skin Symptoms	Exanthema	5 (83)	4 (80)
	Pigmentation	4 (67)	1 (20)
	Desquamation	3 (50)	1 (20)
	Loss of Hair	1 (17)	0 (0)
	Nails Change	5 (83)	2 (40)
Blood Picture	Hb. (80>%)	5 (83)	4 (80)
	R. B. C. (400×10⁴>)	4 (67)	3 (60)
	W. B. C. (7000>)	4 (67)	2 (40)
	Neutrophilic W. B. C. (20%>)	6 (100)	4 (80)
	Eosinophilic W. B. C. (4%<)	4 (67)	2 (40)
Liver Function	Cob. R. >3. 4. 5<	2 (33)	3 (60)
	Cad. R. >6. 7. 8<	1 (17)	2 (40)
	Bromsulfalein Test	4 (67)	3 (60)
Thorn's Test		4 (67)	1 (20)
Changes of E. C. G.		0 (0)	1 (20)

TABLE 17. CHANGES OF CONCENTRATION OF Na AND K IN SERUM OF PATIENTS

		Number of cases	Normal Range Na : 126~140mEq/l K : 3.6~4.8 〃	Na : 125mEq/l> K : 3.69 〃	Na : 140mEq/l< K : 4.8 〃
Na	1st Examination 1/X	19 cases	11 (68%)	8 (42%)	0 (0%)
	IInd Examination 1/XI	10 〃	8 (80%)	0 (0%)	2 (20%)
K	1st Examination 1/X	19 〃	5 (26%)	0 (0%)	14 (74%)
	IInd Examination 1/XI	10 〃	7 (70%)	0 (0%)	3 (30%)

tyrosin, which is a basic material for melanin formation, is lacking, then pigment generation is inhibited. Van Scott and others [36] proved that the -SH group was contained in the water soluble extract of human epidermis, impeding the tyrosin-tyrosinase reaction. Consequently, in the normal melanocyte (or skin), both tyrosinase and the -SH compound which inhibits its activity are assumed to coexist, maintaining an equilibrium. When this -SH compound and arsenic are combined, pigment production is naturally promoted, and pigmentation occurs on the skin. Also, the tyrosinase activity is promoted by ultraviolet rays or high temperature; thus, it is conspicuous on rubbed parts, the outer surface of the genitals, and the parts of the skin exposed to the sun's light. Concerning pigmentation due to the lowering of adrenal function, tracing a cause to compensatory hyperpituitarism was recently considered [37]. It is explained as follows. When the adrenal cortex function weakens and the hormone secretion decreases, this decreased secretion accelerates the pituitary function compensatingly, and melanocyte-stimulating hormone (MSH) and ACTH are secreted in great quantity. Because of this MSH, the function of melanocyte is accelerated. If the adrenal medulla is attacked at the same time, adrenalin, which is known as a substance to inhibit MSH, decreases, the melanocyte function is freely demonstrated, and pigmentation occurs. It is also known that 1/100 mol concentration of arsenic promotes the action of MSH onto melanocyte. It is to be cautioned here that causing hypopituitarism as the primary symptom and lowering of the adrenal function as the secondary symptom suppresses melanin formation. Summarizing the above facts, the pigmentation mechanism of arsenic poisoning is conjectured to be the following:

1) Arsenic damages the adrenal cortex; MSH secretion from the pituitary gland increases secondarily and the melanocyte function is promoted.

2) Arsenic damages the adrenal medulla and suppresses the secretion of adrenalin which suppresses the MSH function. As a result, the melanocyte function is promoted.

3) Arsenic combines with the -SH compound which supresses melanocyte and thus promotes the melanocyte function.

4) Because arsenic poisoning occurred in the summer, the tyrosinase activity in melanocyte was increased. On the other hand, if the stimulus to melanocyte is excessive, causing a reduction in its function, leukoderma could be generated.

TABLE 18. RELATIONSHIP BETWEEN K CONCENTRATION IN SERUM AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

K Concentration in Serum / Clinical Symptoms		5.6mEq/l< 7 cases	5.5mEq~4.9mEq/l 7 cases	4.8mEq~3.6mEq/l Normal 5 cases
Digestive Symptoms		7 (100%)	4 (57%)	4 (80%)
Respiratory Symptoms		4 (57)	4 (57)	2 (40)
Fever		2 (29)	5 (71)	4 (80)
Liver Enlargement		5 (71)	4 (57)	2 (40)
Spleen Enlargement		1 (14)	1 (14)	0 (0)
Skin Symptoms	Exanthema	5 (71)	5 (71)	5 (100)
	Pigmentation	4 (57)	4 (57)	3 (60)
	Desquamation	1 (14)	4 (57)	2 (40)
	Loss of Hair	0 (0)	1 (14)	2 (40)
	Nails Change	4 (57)	4 (57)	4 (80)
Blood Picture	Hb. (80%>)	4 (57)	6 (86)	1 (20)
	R.B.C. (400×10⁴>)	4 (57)	5 (71)	2 (40)
	W.B.C. (7000>)	2 (29)	5 (71)	3 (60)
	Neutrophilic W.B.C. (20%>)	6 (86)	6 (86)	4 (80)
	Eosinophilic W.B.C. (1%<)	0 (0)	5 (71)	2 (40)
Liver Function	Cob. R. >3.4.5<)	4 (57)	2 (29)	2 (40)
	Cad. R. >6.7.8<	4 (57)	1 (14)	1 (20)
	Bromsulfalein Test	4 (57)	3 (43)	3 (60)
Thorn's Test		2 (29)	4 (57)	2 (40)
Changes of E.C.G.		1 (14)	0 (0)	0 (0)

TABLE 19. RELATIONSHIP BETWEEN Na CONCENTRATION IN SERUM AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

		140~126mEq/l 11 cases	125mEq/l> 8 cases	Na : 125mEq/l> K : 4.9mEq/l< 3 cases
Digestive Symptoms		8 (73%)	7 (88%)	3 (100%)
Respiratory Symptoms		6 (55)	4 (50)	2 (67)
Fever		6 (55)	5 (63)	1 (33)
Liver Enlargment (4cm<)		6 (55)	5 (63)	3 (100)
Spleen Enlargement		1 (9)	1 (13)	1 (33)
Skin Symptoms	Exanthema	8 (73)	7 (88)	2 (67)
	Pigmentation	6 (55)	5 (63)	2 (67)
	Desquamation	7 (64)	5 (63)	2 (67)
	Loss of Hair	1 (9)	2 (25)	0 (0)
	Nails Change	5 (45)	7 (88)	3 (100)
Blood Picture	Hb. (80%>)	7 (64)	4 (50)	3 (100)
	R. B. C. (400×10⁴>)	7 (64)	4 (50)	2 (67)
	W. B. C. (7000>)	5 (45)	5 (63)	2 (67)
	Neutrophilic W. B. C. (20%>)	9 (82)	7 (88)	3 (100)
	Eosinophilic W. B. C. (4%<)	4 (36)	3 (38)	1 (33)
Liver Function	Cob. R. >3. 4. 5<	5 (45)	3 (38)	1 (33)
	Cad. R. >6. 7. 8<	5 (45)	1 (13)	0 (0)
	Bromsulfalein Test	7 (64)	4 (50)	1 (33)
Thorn's Test		5 (45)	3 (38)	1 (33)
Changes of E. C. G.		1 (9)	0 (0)	0 (0)

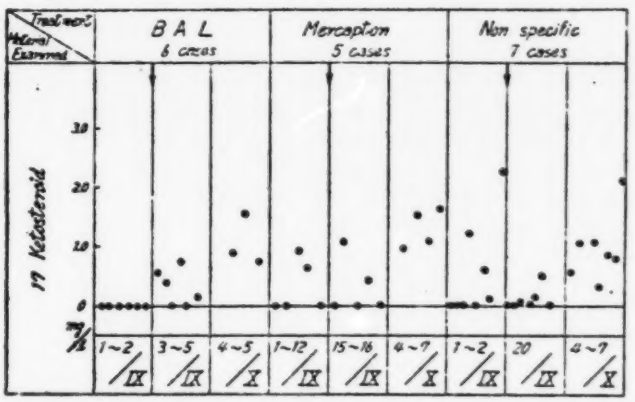

Figure 2. Changes of amount of 17 ketosteriod in urine of in-patients.

TABLE 20. RELATIONSHIP BETWEEN AMOUNT OF 17 KS IN URINE AND CLINICAL SYMPTOMS AT THE FIRST CONSULTATION

Clinical Symptoms \ Amount of 17KS in urine		Omg/l 13 cases	0.11~2.28 mg/l 6 cases
Digestive Symptoms		8 (62%)	6 (100%)
Respiratory Symptoms		5 (38)	5 (83)
Fever		7 (54)	4 (67)
Liver Enlargement		5 (38)	1 (17)
Spleen Enlargement		1 (8)	1 (17)
Skin Symptoms	Exanthema	10 (77)	5 (83)
	Pigmentation	9 (69)	2 (33)
	Desquamation	5 (38)	2 (33)
	Loss of Hair	3 (23)	0 (0)
	Nails Change	8 (62)	4 (67)
Blood Picture	Hb. (80%>)	7 (54)	4 (67)
	R.B.C. (400×10^4>)	7 (54)	4 (67)
	W.B.C. (7000>)	7 (54)	3 (50)
	Neutrophilic W.B.C. (20%>)	11 (85)	5 (83)
	Eosinophilic W.B.C. (4%>)	5 (38)	2 (33)
Liver Function	Coh.R. >3.4.5<	5 (38)	3 (50)
	Cal.R. >6.07.8<	2 (15)	4 (67)
	Bromsulfalein Test	8 (62)	3 (50)
Thorn's Test		5 (38)	3 (50)
Changes of E.C.G.		0 (0)	1 (17)

CONCLUSIONS

In this paper, a study was conducted by comparing the biochemical observations on subacute and chronic arsenic poisoning with the clinical pictures. The summary is as follows:

I. A survey of reports concerning kinds, toxic action, and toxic quantity of arsenic derivatives and the method of arsenic determination is performed.

II. By separating 14 cases with a clearly known amount of arsenic intake into 7 cases, each of more than 10 mg/kg arsenic intake and less than 4.9 mg/kg arsenic intake, respectively, the clinical pictures of these two groups were compared and the following results were obtained:

i) In the group with a larger arsenic intake, all symptoms except for pigmentation were stronger.

ii) The pigmentations were nearly the same in both groups. In other words, each degree of arsenic poisoning is proportional to the amount of arsenic intake, except for pigmentation. Thus, determining the seriousness of subacute or chronic arsenic poisoning just from the pigmentation requires a great deal of caution.

III. Relation Between Arsenic Determination of ~~Each Sample~~ and Clinical Picture

1) Amount of arsenic in blood and clinical picture

The amount of arsenic in the blood of the normal infants is 0-0.3 γ/cc. Its concentration in the blood of affected infants was large, reaching a maximum of 3.1 γ/cc. By comparing the clinical pictures at the first consultation and at the time of arsenic measurement in the blood between the group with a high arsenic concentration in the blood and the group with a relatively low concentration, the following results were obtained:

i) In the group with a high arsenic concentration in the blood, respiratory symptoms, fever, liver enlargement, anemia, and a reduction in white blood cells and neutrophilic white blood cells were frequent; the bromsulfalein test and the Thorn test were also affected.

ii) There was no difference between the two groups in pigmentation, desquamation, leukoderma, and nail changes.

iii) The clinical pictures at the time of arsenic measurement, which took place after some time, differ from those at the first consultation in that the difference in digestive symptoms and the brosulfalein test between groups becomes conspicuous, but the difference in liver enlargement and the change in blood picture is no longer seen.

2) Amount of arsenic in urine and feces and clinical pictures

The amounts of arsenic in the urine and in feces of normal infants were 0-50 γ/1 and 0-5 γ/10 gm dry weight, respectively. The clinical symptoms were compared by separating into groups with a large amount of arsenic in the urine (more than 20 γ/dl) and groups with a small amount (less than 20 γ/dl). Other than the stronger rate of reduction of Hb for the former at the first consultation, no difference was seen in these two groups. The following relations to the clinical picture at the time of arsenic measurement in the urine can be observed. For the former, liver enlargement is small, liver enlargement is small, liver function impediment (cobalt and cadmium reaction) is strong, and adrenal function impediment (Thorn test) is small. For the group with a small arsenic discharge, the opposite behavior is observed. That is, liver function is not greatly impeded, although liver enlargement is strong. The closer the adrenal function is to the normal condition, the better may be the arsenic discharge.

3) Relation between amount of arsenic intake and arsenic discharge and clinical symptoms

The clinical findings were worse when the amount of arsenic intake was large, and the amount of discharge was small.

4) Relation between arsenic in hair and clinical picture

The amount of arsenic in normal infant's hair is 0-50 γ/100 gm. Most patients had more than 1 mg/100 mg, the maximum being 10 mg/100 mg. When separated into two groups depending on the amount of arsenic in the hair, the group with a greater amount of arsenic had a worse clinical picture. However, there was no difference in pigmentation between these two groups. As the amount of arsenic in the hair is not greatly affected by the discharge, it is a good index to indicate the seriousness of symptoms in subacute and chronic arsenic poisoning.

5) No close relation was observed between the clinical picture and the amount of arsenic in nails and in blister fluid obtained by pasting a blister-blaster on the skin

6) Relation between serum protein fraction and clinical picture

Although the patients affected with arsenic poisoning showed a decrease in albumin and an increase in β and γ globulin, no relationship between these changes and the clinical picture was observed. There was a serious case in which a liver enlargement of 8 cm resulted, even though the serum protein fraction was very close to normal. This suggests a very complex relationship between arsenic poisoning and the internal organs which produce the serum protein fraction.

7) Relation between serum -SH group concentration and clinical picture

The serum-SH goes down during arsenic poisoning. A rather good clinical picture was seen in the group with lowered serum-SH. We like to think that this is because of the defensive action of the serum-SH group against toxic material (arsenic) invading the tissues.

8) Relation between Na and K concentrations in serum and clinical picture

In the serum of patients, the low Na and high K symptoms were seen at first, but they recovered to within the normal range later. Comparing the K concentration in the serum and the clinical picture, the clinical picture tended to be worse for a medium high value of K. The change in electrocardiogram appearing during arsenic poisoning is not due to hypopotassemia. Rather, from the viewpoint of K value only, hyperpotassemia is though to be related. No distinct difference was observed between the Na concentration in the serum and the clinical picture.

9) Relation between 17 KS in urine and clinical picture

The lowering of the adrenal function was indicated at first, but this function was accelerated later. In the group with a small amount of 17 KS in urine, liver enlargement and pigmentation were strong.

10) Adrenal function and arsenic poisoning

The behaviors of serum-SH, Na and K in serum, and 17 KS in the urine of patients affected with arsenic poisoning indicate that arsenic poisoning affects the adrenals. Therefore, the relation between arsenic poisoning and adrenals and the pituitary body, as well as the machanism for generating pigmentation during arsenic poisoning, were discussed.

REFERENCES

1. Nagai, H. Shonika Kiyo, 1: 228, 1955.
2. Woody, N.C. and J.T. Kometani. Pediatrics. 1: 372, 1948.
3. Mühlens, P. Dtsch. Med. Wshr.; 58Jg: 854, 1932.
4. Hughes, R.R. Brit. Med. J., 2: 921, 1952.
5. Heffter, A. Handb. d. Exp. Pharm. III Vol. Berlin. Verlag von Julius Springer. 1937, p. 162.
6. Brock, J. Biol. Dat. f.d. Kinderzt. II Edition. II Vol. Springer-Verlag 1954, p. 311.
7. Heffter, A. and W. Heubner. Handb. d. Exp. Pharm. Vol. III, 1 half. 1927, p. 479.
8. Jacobs, M.B. The Analyt. Chem. of Ind. Pois. Haz. & Solv. Vol. 1 Interscience Publishers New York, London. 1949, p. 233.
9. Sandell, E.B. Col. Det. of. Traces. of. Metal. II Edition Interscience Publishers New York, London. 1950, p. 175.
10. Kingsley, G.R., et al. Anal. Chem 23: 914, 1937.

11. Iwataki, N., et al. Hanshi Idaishi, 3: 65, 1953.

12. Williams, M. Trace Elements in Blood 1949.

13. Chapman, A.C. Analyst 51: 548, 1926.

14. Mattice, M.R., et al. A.J.M.S., 193: 413, 1937.

15. Mayers, M.R. Arch. Indust. Hyg. occup. Med. 9: 384, 1954.

16. Kingsley, G.R. et al. Anal. Chem., 23: 914, 1951.

17. Neal, P.A. Publ. Health. Bull., 267, 1941.

18. Künkele, F. Chem. Ztg., 64: 29, 37, 1940.

19. Johnstone, R.J. Ocup. Med & Ind. Hyg., St Louis, 1948, p. 289.

20. bang, I. Biochem. Z., 1965: 364, 1925.

21. Tate, M. Hokkaido Rodo Kenkyu, 59: 31, 1955.

22. Kölsch, F. Lehrb. d. Arbeitshyg. Vol. 1, Ferdinand Enke Verlag Stuttgart. 1947, p. 250.

23. Seipō. Hoppe Seyler., 267: 29, 1941.

24. Kölsch, F. Lehrb. d. Arbeitshyg. Vol. 1, Ferdinand Enke Verlag Stuttgart. 1947, p. 222.

25. Johnstone, J.R. Occup. Med & Ind. Hyg., St Louis, 1948, p. 82.

26. Lewis. Am. J. physiol., 164: 61, 1951.

27. Barron, E.S.G. Advances in Enzymol. XI; 201, 1951.

28. Shacter, B. and C. Entenman. Am. J. Phys., 170: 442, 1952.

29. Shoenbach, E.B., et al. Proc. Soc. Exp. Bio & Med., 73: 44, 1950.

30. Surawicz, B. and E. Lepeschkin. Circulation, 8: 801, 1953.

31. Winkler, A.W., et al. Am. J. Phys. 124: 478, 1938.

32. Lenzi, F. et al. On the Nature of the Myocardial Contraction Korger, New York, 1953, p. 71.

33. Miki, S. Not yet reported.

34. Ujiie, T. Seiikaishi, 60: 1361.

35. Fitzpatrick, T.B., et al. Arch. Derm., 69: 133, 1954.

36. Van Scott, E.J. et al. J. Invest. Derm., 65: 111, 1953.

37. Klinefelter, J. Clin. End & Met., 13: 624, 1953.